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Structural Studies of Prokaryotic RNA Polymerase

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Beamline(s): X25, X9A

Introduction: Transcription is the major control point of gene expression and RNA polymerase (RNAP) is the central enzyme of transcription. Our long term goal is to understand the mechanism of transcription and its regulation. Determining three-dimensional structures of RNAP and its complexes with DNA, RNA, and regulatory factors is an essential step. This is best accomplished with highly characterized prokaryotic RNAPs, especially because of the high degree of conservation of RNAP structure and function from bacteria to man.

To this end, we determined the 3.3 Å-resolution crystal structure of a prokaryotic RNAP, the 380 kDa core RNAP from the thermophilic eubacteria *Thermus aquaticus* (Taq; subunit composition $\alpha_2\beta\beta'\omega$) and employed extensive crosslinking experiments to construct a model of the ternary elongation complex containing core RNAP, DNA template, and RNA transcript (1-3). We also solved the co-crystal structure of RNAP with rifampicin, an important antibiotic inhibitor (2). Our current work is aimed towards adding to our understanding of the enzyme's function and its regulation.

Results: We have two main lines of research, one on the RNAP itself (conducted mainly at X25), and on the promoter specificity σ factors (conducted mainly at X9A):

Structural studies of RNAP

1. We are determining the 3.0 Å resolution crystal structure of Taq core RNAP complexed with nucleotide substrates.

2. We are determining the crystal structures of Taq core RNAP complexed with several antibiotic inhibitors.

3. We have determined the crystal structure of the 430 kDa Taq RNAP holoenzyme (core RNAP plus the 50 kDa Taq σ^A). The best crystals so far are around 0.2 mm x 0.1 mm, but so thin in the 3rd dimension that it is unmeasurable. Despite this, we've collected native data to 4 Å and a derivative data set with a metal cluster (Ta_6Br_{14}) to 4.5 Å. We've obtained an excellent map by combining phases from molecular replacement and from the Ta derivative. We are working to improve the crystal morphology (thickness) to get better resolution.

5. We are determining the crystal structure of Taq RNAP holoenzyme complexed with promoter DNA fragments. We have solved one crystal form to 6.5 Å resolution. Another crystal form diffracts isotropically to better than 4.5 Å.

Stuctural studies of RNAP σ factors

1. We have solved crystal structures of the domains of Taq σ^A that span the important regions of the molecule (ranging from 2.6 to 1.8 Å resolution), along with the 2.6 Å-resolution structure of one domain in complex with a conserved promoter fragment (corresponding to the –35 element). This work has been submitted for publication (4).

2. We have solved the 2.9 Å-resolution crystal structure of a complex between an intact σ factor (the 29 kDa σ^F from *Bacillus stearothermophilus*) and its cognate regulatory molecule, the anti-σ factor SpoIIAB (a dimer of 16 kDa subunits), which is also a serine kinase. This work has been submitted for publication (5).

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